PN-ABS-457

FINAL REPORT

Covering Period:	September 1991 to August 1994
	Submitted to the Office of the Science Advisor U.S. Agency for International Development
Project:	Method to Assess Vitamin A Status in Malnourished Children
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Project No.:	10.293
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AID Grant Project Officer:	Dr. Francis Davidson
Project Duration:	Sept. 1991 to Aug. 1994

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3) Executive Summary:

Vitamin A (Vit. A) deficiency (xerophthalmia) is estimated to affect over five million children and to blind about half of them each year in Asia alone. One of the most important consequences of Vit. A deficiency, in addition to the permanent blindness and impaired growth, is the increased rate of infection and associated morality in pre-school children in the underdeveloped countries. As a result of these epidemiological studies, numerous intervention programs that include Vit. A supplementation or fortification are presently underway in various parts of the world. In order to conduct relevant research projects and develop reliable and targeted public health nutrition programs it is very necessary to assess Vit. A status in malnourished children. Since plasma levels of Vit. A do not necessarily reflect its status, there is a need to develop accurate and relatively simple methods to assess it. The relative dose response (RDR) method, although measures liver stores of Vit. A, requires two blood samples, five hours apart, which pose an ethical problem particularly for the newly born children of, say, 4-6 weeks old. A new assay using 3,4-didehydroretinol (Vit. A_2) has been introduced in which only one blood sample is required. This method is termed as the modified RDR or MRDR.

In order to implement this new method, i.e. MRDR, for the assessment of Vit. A in malnourished children, Vit. A_2 was synthesized. The synthesis of A_2 following some old literature was not very successful. Due to the lack of timely availability of a reagent from England, A_2 was purchased from Dr. J. Olson's laboratory at the Iowa State Univ., Ames, Iowa. Further details of methods and procedures are given in Section 5 (Methods and Results) below.

Initially, seventy-three malnourished children were recruited through the ICDDR,B Hospital and the MRDR test was formed. However, only fifty of the recruited subjects reported for the RDR experiment. All the results are, therefore, analyzed based on these 50 malnourished children, although some other data for all the children are given in Table 1. While the overall results are described in Section 5 below, the summary of the analysis indicates that the MRDR test, as presently administered, is ineffective in comparison with the RDR test in malnourished children with demonstrated LOW vitamin A stores.

4) Research Objectives:

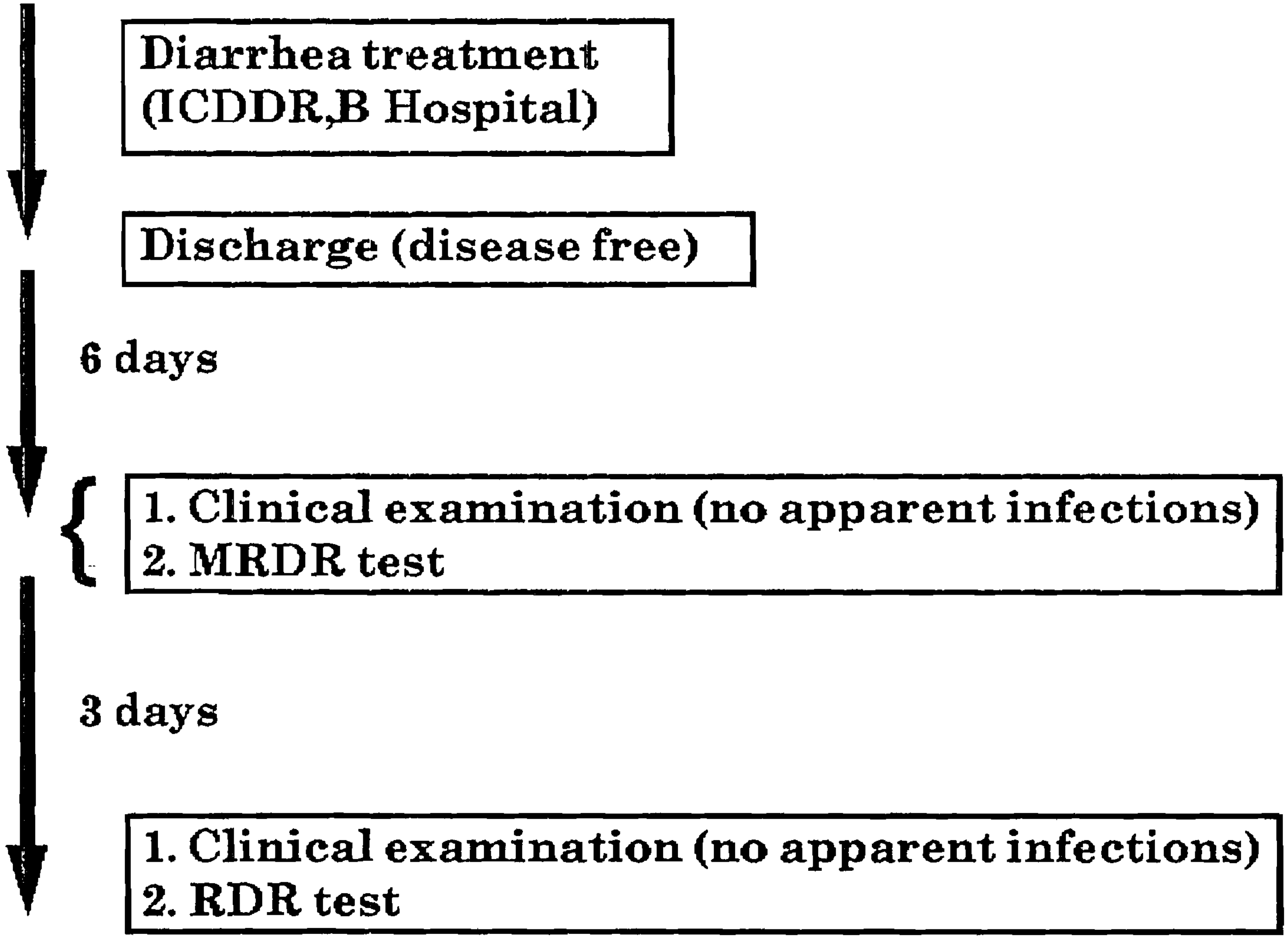
The purpose of this project was to 1) synthesize Vitamin A_2 and 2) use it for the development of MRDR method in order to assess the Vit. A status in malnourished children.

5) Methods and Results:

The study design is shown on page 5 and the subjects selection criteria on page 6. Subjects physical characteristics are presented in Tables 2 to 4. Brief description of experimental methods and procedures are outlined on pages 10, 11 and 12. The HPLC chromatogram for measuring A_1 and A_2 is illustrated in Fig. 1. Serum retinol (A_1), MRDR and RDR data are analyzed and plotted in Figures 2 to 4. Vitamin A status as measured by the MRDR and RDR methods are presented in Tables 5 and 6 respectively. The following conclusions could be made from the data presented:

1. Almost half of the malnourished children had low serum vitamin A, RBP and Prealbumin levels.
2. Sensitivity of the MRDR test to detect low vitamin A store was lower than that of RDR test.
3. MRDR test, as presently administered, may be inadequate to screen vitamin A status in population where malnutrition is prevalent.

STUDY DESIGN



Note: After the RDR test, all children were given a Vitamin A suppleme

STUDY SUBJECTS

INCLUSION

- Children (3-36 m) coming to ICDDR,B hospital after recovering from a recent diarrhoeal episode
- Parental informed consent

EXCLUSION

- Children who have had vitamin A supplementation within last 3 months
- Children with kwashiorkor or with systemic infections

TABLE 2

Characteristics of study children (n=50)

Age (months):			
	Mean	=	16.7
	Median	=	13.0
	Range	=	4.0-34.0
Sex:			
	Male	=	27
	Female	=	23
Body weight (kg):			
	Mean	=	7.3
	Median	=	7.3
	Range	=	3.9-10.5

TABLE 3

Distribution of study children by age (n=50)

Age (m)	Number	%
3- 5	4	8.0
6- 11	13	26.0
12-23	23	46.0
24-35	10	20.0

TABLE 4

Distribution of study children by nutritional status

Wt/age	Number	%
< 60	10	20.0
60-74.9	27	54.0
≥75	13	26.0

METHODS AND PROCEDURES

MRDR

- A dose of (100 µg/kg body weight) 3,4-didehydroretinol acetate (supplied by Iowa State University) was placed on the tongue followed by icecream
- After 5 hours of dose 2 ml blood was drawn by venipuncture for serum Vitamin A_1, Vitamin A_2, RBP and Prealbumin estimation
- Serum was extracted with MEOH/Hexane. A_1 and A_2 were measured by HPLC
- Children with A_2/A_1 ratios ≥ 0.06 were judged vitamin A depleted

RDR

- Two ml blood was drawn by venipuncture in AM.
- A dose of (1000 µg retinol equivalents) Vitamin A Palmitate (Arovit-ROCHE) was poured on the tongue followed by icecream.
- After 5 hours of dose, another 2ml blood was obtained.
- Serum from both the blood was run for Vitamin A by HPLC, RBP and Prealbumin by commercial immunodiffusion plates
- Children with $\geq$20% of (A5-A0/A5x100) were judged Vitamin A depleted

HPLC CONDITION

- WATER's HPLC with
  - Model 510 pump
  - Model 481 detector
  - Model 745 Integrator
  - Model 712 WISP autosampler
  - Mobile phase (MEOH : H_20 = 95:5) 1 ml/min
  - Detector - nm 350 for A_2 and nm 325 for A_1
  - Attenuation at 8 and chart speed 0.5 cm/min

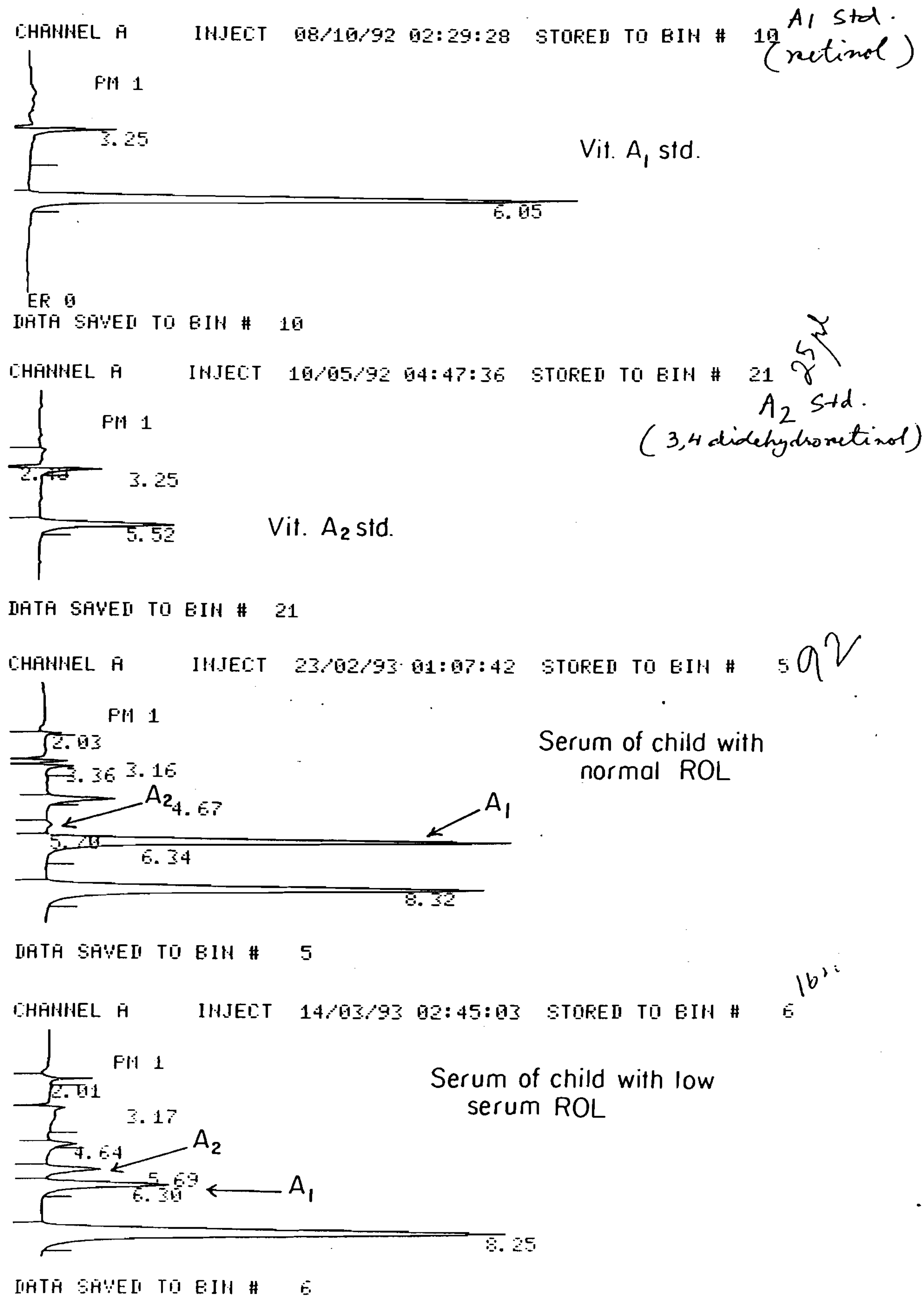

CHANNEL A INJECT 08/10/92 02:29:28 STORED TO BIN # 10
A1 Std. (retinol)
PM 1
3.25
Vit. A1 std.
6.05
ER 0
DATA SAVED TO BIN # 10
CHANNEL A INJECT 10/05/92 04:47:36 STORED TO BIN # 21
A2 Std. (3,4 didehydroretinol)
PM 1
3.25
Vit. A2 std.
5.52
DATA SAVED TO BIN # 21
CHANNEL A INJECT 23/02/93 01:07:42 STORED TO BIN # 5
PM 1
2.03
3.36
3.16
Serum of child with normal ROL
A2
4.67
A1
6.34
8.32
DATA SAVED TO BIN # 5
CHANNEL A INJECT 14/03/93 02:45:03 STORED TO BIN # 6
PM 1
2.01
3.17
Serum of child with low serum ROL
4.64
A2
5.69
6.30
A1
8.25
DATA SAVED TO BIN # 6

FIGURE 1

TABLE 1

ID	Age	Wt	Ht	MAC	Wt/Age	A1 µg/dl	A2 µg/dl	A1 (0h)	A1 (5h)	MRDR	RDR	RBP
1	12	6.62	67	12.6	65.22	15.1	0.072			0.005		
2	6	7	66	13.5	89.22							
3	20	9.3	76.5	14.2	78.49	29.1	0.29	28.9	28	0.010	-3.2	2.4
4	26	7.29	71	11.1	57.21	18.7		26.4	34.68		16.6	1.2
5	5	5.4	61.1	12.5	81.15							
6	23	9.5	84	13.4	76.55	13.95	0.312	14.58	19.86	0.022	26.6	0.97
7	4	3.9	56	11	64.47	4.3	1.083	4.43	20.85	0.252	78.8	0.75
8	4	5.25	60.5	12	86.79	12.4	0.984			0.079		
9	18	8	73	13.4	73.9	21	1.84			0.088		2.25
10	31	10.9	88	13.6	79.52	24.15	0.66			0.027		1.8
11		10.2	83.5	14.2	79.49	31.2	0.89			0.029		2.82
12	18	8.1	73	13	70.63	16.4	1.79			0.109		
13	7	4.9	61.5	11.2	58.76	30	0.83	26	31.25	0.028	16.8	1.97
14	33	10.45	83	13.6	74.23	28.95	0.635	31.7	34.7	0.022	8.6	2.25
15	18	9.3	81	13	81.09	24.6	0.886	25.06	30.3	0.036	17.3	2.4
16	25	10.25	82.2	14.2	81.71	11.5	0.638	21	29.2	0.055	28.1	1.35
17	12	7.25	70.5	13.6	76.06	14.6	0.425	16.2		0.029		
18	13	6.8	67	14.2	65.31	25.3	0.31	26.6	39	0.012	31.8	2.7
19	12	6.35	67.5	12.6	66.62	16.1	0.345			0.021		
20	33	9.5	83.2	12.8	69.95	25	0.509	29.5	37.7	0.020	21.8	2.75
21	33	10.35	84.5	14.4	76.21	41.78	1.54	40.1	43.6	0.037	8.0	3
22	25	10.5	84.5	14	87.4	25.3	0.376	28.95	35.2	0.015	17.8	2.4
23	8	5.4	65	11	61.49	1.7	1	2.74	21.75	0.588	87.4	1.15
24	22	8.4	74.5	12.8	72.73	51.5	0.795	48	46.9	0.015	-2.3	3.95
25	13	7.4	75.5	14.2	71.07	23.4	0.868	24.2	41.2	0.037	41.3	1.85
26	12	5.25	65	10.8	55.08	11.37	1.81			0.159		1.6
27	31	8.3	78.2	12	62.81	14	0.312			0.022		1.35
28	24	7.6	72.5	12.8	64.43	12.9	1.068			0.083		
29	10	7.2	69.9	13.4	75.48	27.46	0.509	37.15	45.6	0.019	18.5	2.15
30	6	6.62	64	13.4	91.84	27.2	1.216			0.045		
31	22	8.2	74.5	14	67.08	28.2	1.27	46.2	53.6	0.045	13.8	2.75
32	22	6.9	74	10.4	59.75	9.16	3.37	6.8	30.8	0.368	77.9	2.15
33	20	6.08	66	11	51.32	12.7	1.718	16.25	33.2	0.135	51.1	2.05
34	14	7.3	71	12.4	68.53	18.8	0.352	29.1	38.3	0.019	24.0	1.77
35	18	8	75	12.4	69.76	17.3	1.058	24.1	30.2	0.061	20.2	1.85
36	33	9.37	82	14.2	68.99	27.7	0.595	44.2	58.5	0.021	24.4	2.75
37	11	6.07	68	11.8	65.68	14.3	0.093	19.2	34.8	0.007	44.8	1.3
38	9	5.98	63.2	12.4	69.86	15.1	0.522	8.15	24.3	0.035	66.5	1.85
39	15	7.4	74.5	13	68.06	24.1	0.141	33.5	43.7	0.006	23.3	2
40	11	5.02	63	11	54.31	15.74	0.745	13.4	26.87	0.047	50.1	1.35
41	12	5.7	64	11	56.16	25		40.7	53.3		23.6	1.6
42	10	5.8	65	11.5	65.02	25.4	0.548	21	26.2	0.022	19.8	2.15
43	15	6.5	68	12.2	63.44	11.8	0.478	15.7	31.1	0.041	49.5	1.1
44	26	7.52	76	11.2	61.51	26	0.96	29.6	39.4	0.037	24.9	3
45	13	8.48	72.3	13.2	81.44	6.8	0.279	17.14	24.04	0.041	28.7	0.92
46	18	7.57	73.1	12.8	69.93	40.25	0.413	36.12	39.2	0.010	7.9	4.5
47	5	4.3	60.5	10.4	58.93	28.7	0.239	30.3	47.3	0.008	35.9	
48	7	5.45	64	11.6	65.36	16.88	0.232	26.92	45.85	0.014	41.3	2.2
49	8	6.82	66	11.8	77.66	15.23	0.5			0.033		
50	4	3.95	55	9.6	65.3	5	0.874	5.09	20.2	0.175	74.8	1.45
51	7	6.4	64	12.2	76.75	20.85	0.911	22.3	30.8	0.044	27.6	2.32
52	15	6.83	70.1	10.8	62.82	17.7	0.895	19.8	23.46	0.051	15.6	2.03

TABLE 1(contd.)

53	10	8.53	73	13.2	89.42	36.5	0.868			0.024		
54	16	7.3	71.1	12.2	69.87	21.8	0.749	31.55	39.6	0.034	20.3	2.32
55	5	6.03	61.4	13.8	90.62	26.4	0.556			0.021		
56	18	8.58	75.3	13.6	79.26	24.4	0.5456	27.6	39.7	0.022	30.5	3
57	14	7.3	69.5	13.6	72.79	12.44	0.773	15.2	26.9	0.062	43.5	1.75
58	23	7.62	78	12.5	64.98	23.7	0.388			0.016		
59	8	6.65	67.3	12.5	75.72	27.3	0.255	36.3	41.24	0.009	12.0	2
60	9	7.93	71.4	13.4	86.37	23.2	0.8	24.3	30.2	0.034	19.5	2
61	13	6.07	64.3	12.4	61.99	9.34		25.47	35.1		27.4	1.1
62	13	6.83	72.4	12	65.59	9.2	1.49	13.3	26.4	0.160	49.6	1.45
63	26	7.39	74.5	11.8	57.99	17.1	0.318	27.65	40.3	0.020	31.4	1.85
64	34	8.09	78.3	12.2	58.8	39.67	0.643	33.2	46.8	0.020	29.1	3.65
65	18	8.54	75.5	14	78.89	35.5	0.568	33.5	37.45	0.020	10.6	2
66	6	5.16	60.4	11.6	65.77							
67	13	5.76	65.9	11.6	58.82	27.3	0.466	18.5	19.5	0.020	5.1	1.85
68	21	7.57	70.1	12.8	62.89	17.3	0.961	22.75	30	0.060	24.2	1.85
69	11	7.13	69.8	12.2	72.31	16.3		17	22.7		25.1	1.35
70	5	3.51	55	9	52.75			31.56	45		29.9	1.47
71	9	6.34	67.2	13	74.07	22	0.156	41.8	49.2	0.010	15.0	1.85
72	8	6.55	70.3	12.4	74.58	14.4	0.292	9.7	11.5	0.020	15.7	
73	22	6.9	72	12.2	59.75	9	0.277	12.7	16.9	0.030	24.9	0.77

FIGURE 2

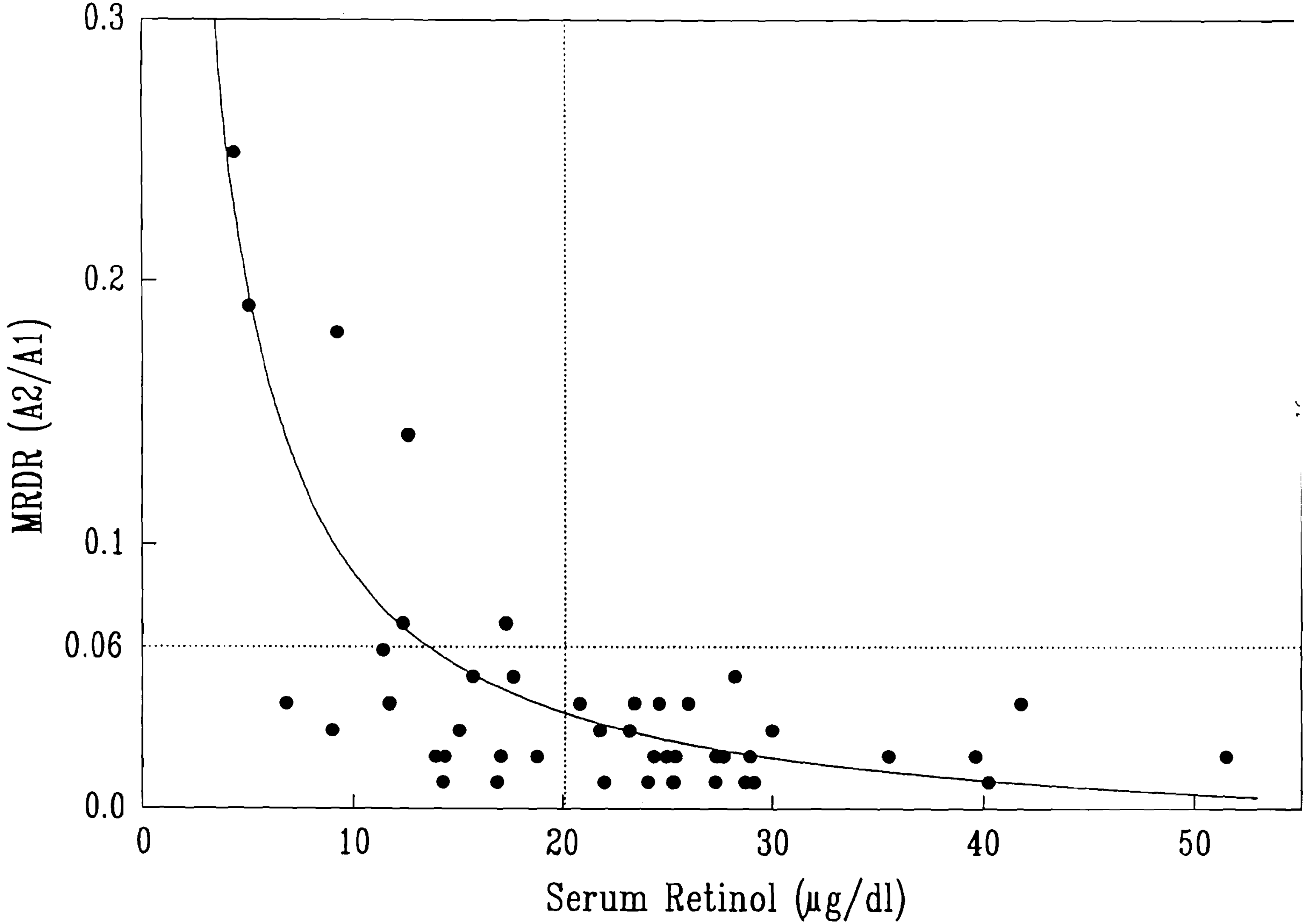


FIGURE 3

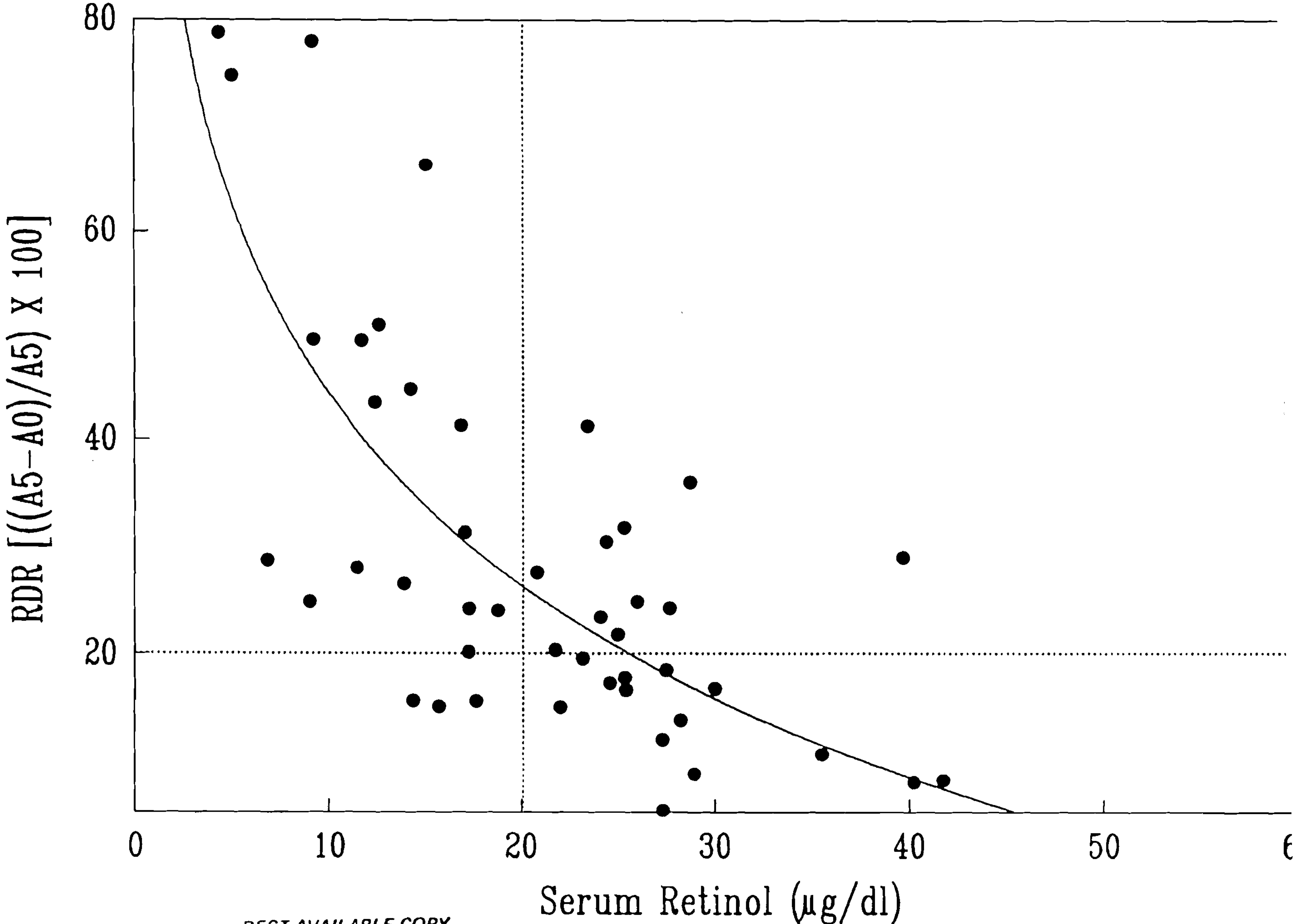


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FIGURE 4

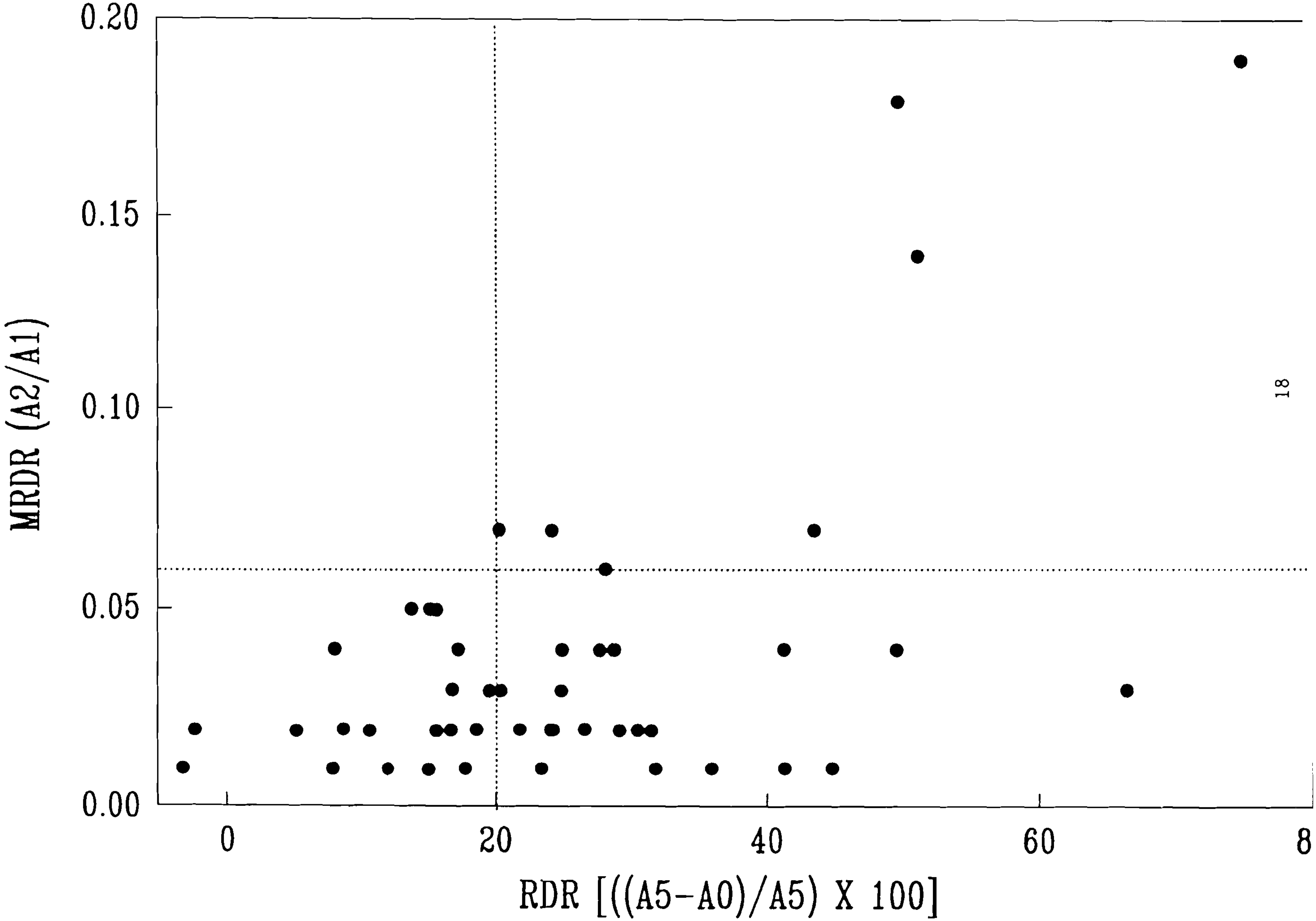

TABLE 5

Distribution of children by vitamin A status measured by MRDR

Serum Vit. A (µg/dl)	Number (%)	MRDR+ve (≥0.06)
≤10	7 (14)	5 (71)
11-19	16 (32)	5 (31)
≥20	27 (54)	0 (0)

TABLE 6

Distribution of children by vitamin A status measured by RDR

Serum Vit.A (μg/dl)	Number (%)	RDR +ve (≥20)
≤10	6 (12)	5 (83)
11-19	12 (24)	10 (83)
≥20	32 (64)	16 (50)

6) Impact, Relevance and Technology Transfer:

The development of a new and accurate method to assess vit. A stores in malnourished children is highly warranted. MRDR is considered an improvement over the so-called RDR method, because it requires only one blood sample after dosing a certain amount of Vit. A_2. The results of the present study indicate that the MRDR does not show any improvement over RDR in the mild to moderately malnourished children. Further studies are needed as suggested below in Section 9 (Future Work). The Bangladeshi research workers received various training and exposure to modern methods and procedures and now they are capable to carry out Vit. A assessment methodologies quite confidently.

7) Project Activities/Outputs:

Dr. Mahalanabis at ICDDR,B, involved in this project, attended the IVACG meeting held in Tanzania in 1993. Dr. Bashar came to UAB and was trained in the synthesis of Vit. A_2. Dr. Khaled received a training on MRDR in Dr. Olson's laboratory at Iowa State Univ., Ames, Iowa in 1991. Mr. Wahed, a research technician in Bangladesh, came to UAB to receive further training on both the synthesis and MRDR technique.

The results have been abstracted into the following titles and were presented in the FASEB meeting held at Anaheim, California, in April this year.

1. Large-Dose Vitamin A Supplementation to Malnourished Children. Abstract #892.
2. Comparison of MRDR and RDR Tests in Assessing Vitamin A Stores in Malnourished Children. Abstract #2555.
3. The Modified Relative Dose Response (MRDR) is Highly Dependent on Percent Saturation of RBP. Abstract #4739.

At least two full length research articles are in preparation.

8) Project Productivity:

The study as designed went very well, although some difficulties due to the usual rules and regulations in the Third World countries delayed the progress of the work.

9) Future Work:

The MRDR method should further be validated using higher doses of Vit. A_2 and perhaps lowering the cut-off point (0.06). The method should also be validated on children before and after deworming since intestinal parasites are largely responsible for the malabsorption of many nutrients, particularly Vit. A (Mahalanabis et al. Am J Clin Nutri 29:1976; 1372-1375).